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Nosocomial ventriculitis caused by a meticillin- and linezolid-resistant clone of *Staphylococcus epidermidis* in neurosurgical patients

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SUMMARY

Background: Postneurosurgical ventriculitis is mainly caused by coagulase-negative staphylococci. The rate of linezolid-resistant *Staphylococcus epidermidis* (LRSE) is increasing worldwide.

Aims: To report clinical, epidemiological and microbiological data from a series of ventriculitis cases caused by LRSE in a Spanish hospital between 2013 and 2016.

Methods: Cases of LRSE ventriculitis were reviewed retrospectively in a Spanish hospital over a four-year period. Clinical/epidemiological data of the infected patients were reviewed, the isolates involved were typed by pulsed-field gel electrophoresis (PFGE) and multi-locus sequence typing, and the molecular bases of linezolid resistance were determined.

Findings: Five cases of LRSE ventriculitis were detected. The patients suffered from cerebral haemorrhage or head trauma that required the placement of an external ventricular drain; spent a relatively long time in the intensive care unit (ICU) (10–26 days); and three out of the five patients had previously been treated with linezolid. All LRSE had the same PFGE pattern, belonged to ST2, and shared an identical mechanism of linezolid resistance. Specifically, all had the G2576T mutation in the V domain of each of the six copies of the 23S rRNA gene, together with the Q136L and M156T mutations and the 71GGR72 insertion in the L3 and L4 ribosomal proteins, respectively.

Conclusion: The high ratio of linezolid consumption in the ICU (7.72–8.10 defined daily dose/100 patient-days) could have selected this resistant clone, which has probably become endemic in the ICU where it could have colonized admitted patients. Infection control and antimicrobial stewardship interventions are essential to prevent the dissemination of this difficult-to-treat pathogen, and to preserve the therapeutic efficacy of linezolid.

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Introduction

Nosocomial ventriculitis is a severe complication associated with invasive procedures such as neurosurgery, complicated head trauma and foreign body placement, since disruption of the biological barriers of the patient can allow direct entry of the pathogen [1]. Insertion of cerebrospinal fluid (CSF) external drains are among the most common invasive procedures in this setting, and are frequently used for the treatment of hydrocephalus and infections of the central nervous system. The incidence of catheter-related CSF infection is variable (1–18%) depending on placement location (e.g. internal ventricular, external ventricular or ventriculoperitoneal) and additional risk factors [1].

Postneurosurgical meningitis and drain-associated infections are usually caused by bacteria from the patient's own skin microbiota, mainly coagulase-negative staphylococci (CNS) such as *Staphylococcus epidermidis*, which is the most common pathogen associated with these types of infection [1,2]. Nosocomial isolates of *S. epidermidis* are often resistant to the majority of antimicrobials available [2,3]. Most studies report a prevalence of meticillin resistance of approximately 75% in this species, which is often accompanied by resistance to many other antimicrobial agents belonging to different families [4]. In addition, this species can adhere to foreign device surfaces due to its ability to form biofilms, which protect the enclosed bacteria, hence hindering the action of antimicrobial agents [3]. Catheter removal and vancomycin therapy are the first-line recommendations for meticillin-resistant staphylococcal shunt-associated ventriculitis. However, vancomycin has poor diffusion in CSF, especially in meningitis without inflammation of the blood–brain barrier. Furthermore, vancomycin toxicity must also be considered in critically ill patients [1]. In addition, *S. epidermidis* isolates with reduced susceptibility to glycopeptides have been reported in recent years [2]. Consequently, linezolid (LZD) is increasingly being used for the treatment of drain-associated ventriculitis due to its good CSF penetration, and efficacy against staphylococcal infections of the central nervous system [1,2].

LZD is an oxazolidinone with activity against Gram-positive micro-organisms, including meticillin-resistant staphylococci and vancomycin-resistant enterococci. It binds to the peptidyl transferase centre (PTC) of the bacterial ribosome, thereby blocking protein synthesis at the initial stage [4]. LZD resistance is increasing worldwide. In Spain, the rate of LZD-resistant *S. epidermidis* (LRSE) in intensive care units (ICUs) was 25% in 2015, according to the ICU-acquired infection Spanish study ENVIN-HELICS [4]. Mutations in the V domain of the 23S rRNA at the PTC constitute the major mechanism of LZD resistance. Alterations in ribosomal proteins L3, L4 and L22, encoded by the *rplC*, *rplD* and *rplV* genes, respectively, have also been associated with oxazolidinone resistance [4,5]. Moreover, the transferable *cfr* gene, which encodes a 23S rRNA methyltransferase, confers resistance to LZD and other antimicrobial families, such as phenicols, lincosamides, pleuromutilins and streptogramin A [4,5], and the *optrA* gene encodes a newly described transferable mechanism of oxazolidinones and phenicol resistance, which provides active efflux of these antimicrobial compounds mediated by ABC transport [6]. All the aforementioned mechanisms of LZD resistance, except *optrA*, have been reported previously in LRSE.

Although several studies have investigated LRSE of clinical origin, only a few have included isolates recovered from CSF, without giving details on the clinical features of the infected patients [2,4]. The present study reports a series of nosocomial ventriculitis caused by a meticillin- and LZD-resistant *S. epidermidis* clone in a Spanish hospital, comprising clinical and epidemiological data of the infected patients as well as the microbiological features of the isolates involved. To the authors' knowledge, this is the first series of nosocomial ventriculitis by LRSE reported to date.

Materials and methods

CSF cultures from hospitalized patients admitted to the Hospital Universitario Central de Asturias (HUCA), Spain, in 2013–2016, and positive for *S. epidermidis* were reviewed retrospectively. The medical records of the patients were also reviewed. Isolates with LZD minimal inhibitory concentration (MIC) >4 mg/L were considered to be resistant, according to Clinical and Laboratory Standards Institute breakpoints [7], and included in the study. Data on LZD utilization were obtained from the hospital pharmacy and expressed as defined daily dose (DDD) per 100 patient-days of bed occupancy.

Bacterial identification and antimicrobial susceptibility testing were performed by the MicroScan System (Beckman Coulter, Porterville, CA, USA). Identification was confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (microflex; Bruker Daltonik GmbH, Bremen, Germany). MICs to LZD and tedizolid were determined using E-test strips (bioMérieux, Marcy l'Etoile, France).

Chromosomal DNA was extracted with Automated Nucleic Acid Extractor MagCore HF16 (RBC Bioscience Corp., New Taipei City, Taiwan), and polymerase chain reaction (PCR) amplification of the six copies of the 23S rRNA gene of *S. epidermidis* was performed as described previously [5]. Nested PCR amplification of the central loop of the V domain of the six copies of the 23S rRNA gene (*rrlA* to *rrlF*), as well as amplification of the *rplV* gene (coding for ribosomal protein L22), were done as reported [5]. For amplification of the *rplC* and *rplD* genes (for ribosomal proteins L3 and L4, respectively), the following primer pairs were designed for the present study: L3-Fw (CATCGTTTAATTCAACTGAACCTG), L3-Rv (CTTACCATCAGGTGTAGACATCGA), L4-Fw (GCACGAGTATCTACATCGAAAGTG) and L4-Rv (AAAGGCAATGTACCTGGACCTA). The obtained amplicons were sequenced and compared with the corresponding genes of the LZD-susceptible *S. epidermidis* reference strain RP62A (ATCC 35984). Screening of *cfr* and *optrA* was also accomplished by PCR [4,6].

PFGE with *Sma*I and multi-locus sequence typing (MLST) were performed as reported previously [8].

Results

Over the study period (2013–2016), 30 *S. epidermidis* were isolated from CSF at HUCA. Seven of these (23.3%) were resistant to LZD, but only five could be recovered for further characterization. LZD and tedizolid MICs for these isolates were >256 mg/L and >32 mg/L, respectively, and all five isolates were also resistant to meticillin but susceptible to vancomycin. The five CSF infections were acquired nosocomially (>48 h after admission) [9], and confirmed as ventriculitis

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Table I
Clinical features of patients with nosocomial ventriculitis caused by linezolid (LZD)-resistant *Staphylococcus epidermidis*

Patient	Sex/age (years)	Date of bacterial isolation[a]	Ward	Length of ICU stay (days)	Underlying condition	Time between EVD implantation and infection (days)	Previous LZD use (no. of days of use)	Treatment/ outcome
1	Male/50	25th July 2013	ICU	17	CH	17	No	Vancomycin IV/ discharged
2	Male/64	29th July 2014	ICU	10	HIV/CH	9	No	Vancomycin IV/ died[c]
3	Male/70	10th July 2015	ICU	16	DM/CH	16	Yes (3)	Vancomycin IV/ discharged
4	Male/42	2nd September 2015	ICU	26	CH	19	Yes (19)	Vancomycin IV and IT/discharged
5	Male/86	1st February 2016	Neurosurgery	23	CLL/HT	4[b]	Yes (7)	Vancomycin IV and IT/discharged

ICU, intensive care unit; CH, cerebral haemorrhage; HIV, human immunodeficiency virus infection; DM, diabetes mellitus; CLL, chronic lymphoid leukaemia; HT, head trauma; EVD, external ventricular drain; IV, intravenous; IT, intrathecal.

[a] *S. epidermidis* isolates recovered from Patients 1–5 were termed *Sep*-HUCA1 to *Sep*-HUCA5, respectively. Two other isolates, which were not available for further characterization, were obtained in April 2013 and March 2015 from patients admitted to the ICU and postoperative ICU, respectively. The first patient was treated with LZD for 12 days before LRSE (LZD-resistant *S. epidermidis*) isolation. The second patient did not receive LZD therapy previous to the LRSE infection.

[b] Patient had multiple previous EVD removals.

[c] The patient died of heart failure unrelated to the infection.

according to Centers for Disease Control and Prevention definitions [10]. All the infected patients suffered from cerebral haemorrhage or head trauma, required external ventricular drain placement, and had prolonged stays in the ICU (10–26 days), and three of them had received LZD therapy previously (Table I). These facts are known risk factors for selection of LRSE [11]. The use of LZD at HUCA was 7.72, 8.10, 8.10 and 7.91 DDDs/100 patient-days in the ICU, and 0.99, 1.21, 2.33 and 2.49 in other hospital wards, in 2013, 2014, 2015 and 2016, respectively.

All isolates shared the same mechanism of LZD resistance. First, they had the G2576T nucleotide mutation (*Escherichia coli* numbering will be used throughout) in the V domain of each of the six copies of the 23S rRNA gene. Second, the Q136L and M156T substitutions in ribosomal protein L3, and the 71GGR72 insertion in ribosomal protein L4 were also detected in all isolates. Finally, all of them displayed the previously described L94V silent mutation in L3 (L101V for *S. epidermidis* ATCC 12228), which does not confer LZD resistance [12], and were negative for the transferable *cfr* and *optrA* genes and wild type for the *rplV* gene (ribosomal protein L22). In addition to sharing the same mechanism of LZD resistance, the five LRSE showed identical PFGE profiles (Figure 1) and were assigned to clone ST2 by MLST.

Discussion

Between 2013 and 2016, seven isolates, all recovered from CSF and involved in ventriculitis, were resistant to LZD, which is an important choice for the treatment of this type of infection. Although the cases reported in this study occurred between 2013 and 2016, the rate of LRSE in the ICU increased drastically in 2012 (from 17% in 2011 to 27% in 2012), and has remained endemic since. LRSE isolates were associated with different types of infection, including ventriculitis and bacteraemia.

The five isolates available for the present study showed the most frequent mechanism for LZD resistance, namely the G2576T mutation in the V domain of the 23S rRNA gene. This change was found in all copies of the gene, which is enough in itself to justify the high MICs observed for LZD and tedizolid in the present study, due to 'gene dosage' as described previously [2,4,5,13]. Nevertheless, ribosomal protein mutations were also found in all isolates, consistent with the complexity of the genetic bases for LZD resistance in *S. epidermidis* [14].

The five isolates belonged to the same ST2 clone, which could have become endemic in the hospital. ST2 is mainly related to hospital care [3], and its ability to acquire and accumulate mutations in the 23S rRNA has been highlighted [15]. LRSE isolates pertaining to ST2 have been associated with outbreaks and long-term endemic situations in hospitals of different European countries, including Spain, as well as in the USA and other American countries [11,12,15–20]. However, none of the LRSE ST2 isolates were obtained from CSF and only a single case of meningitis caused by LRSE has been reported [2]. The latter study focused on daptomycin plus trimethoprim/sulfamethoxazole therapy, rather than clinical and microbiological features, and the sequence type of the isolate was not determined. In fact, to the best of the authors' knowledge, the present study reports the first cases of nosocomial ventriculitis by LRSE to date.

Some authors have suggested low variability of nosocomial *S. epidermidis* and a long temporal persistence of the ST2 clone, in contrast to the high diversity of *S. epidermidis* clones found in the community [3,12]. In agreement with this, the same clone was detected in the five patients admitted to HUCA over the four-year study period (2013–2016), although the hospital moved to a new building in June 2014. A retrospective study of the first available LRSE isolates that were recovered between June 2013 and June 2014 in the old building showed PFGE patterns identical or closely related to that reported in the present study, and also belonged to the ST2 clone (data not

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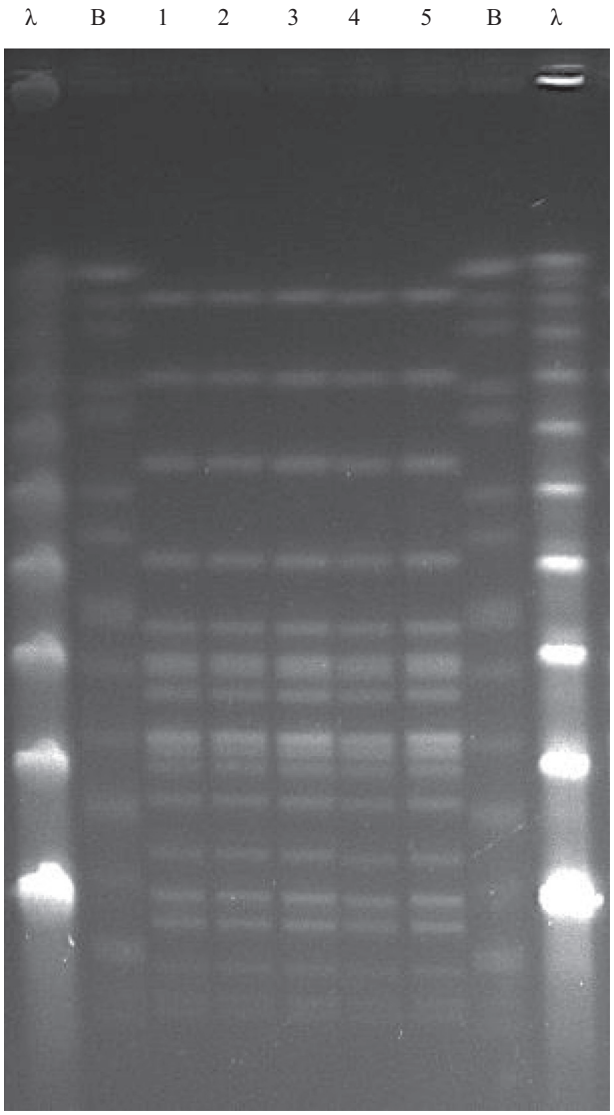

Figure 1. Pulsed-field gel electrophoresis profiles generated from *Staphylococcus epidermidis* isolates by *Sma*I digestion. Lane λ, Lambda Ladder PFG Marker (New England Biolabs, Beverly, MA, USA); Lane B, DNA from *Salmonella enterica* serovar Braenderup H9812 digested with *Xba*I; both used as size standards. Lane 1, *Sep*-HUCA1; Lane 2, *Sep*-HUCA2; Lane 3, *Sep*-HUCA3; Lane 4, *Sep*-HUCA4; Lane 5, *Sep*-HUCA5.

published). According to Widerström *et al.*, the endemic situation of multi-drug-resistant nosocomial clones of *S. epidermidis* mirrors antimicrobial use in hospitals, especially in ICUs [3]. Strong antimicrobial pressure can select these clones and lead to rapid colonization of the skin of hospitalized patients [21]. The high consumption of LZD in the ICU of HUCA (7.72–8.10 DDDs) approximates the 13 DDD threshold considered by other authors as necessary for the occurrence of an outbreak of LZD-resistant bacteria [4]. Cross-transmission and high use of LZD in the ICU could have played a key role in the establishment of the ST2 LRSE clone at HUCA, increasing the risk of nosocomial drain-associated infections and decreasing therapeutic options. In fact, the isolates studied here were only susceptible to vancomycin, teicoplanin, daptomycin, fosfomycin and tetracycline, thus complicating antimicrobial therapy. Although all patients were treated with vancomycin and all except one (who died due to heart failure unrelated to the LRSE infection) were discharged, the therapeutic options would have been extremely limited in the case of glycopeptide intolerance.

Infection control measures have been established over recent years in the ICU of HUCA due to the emergence of LRSE and the severity of the infections it causes, such as those reported in this study. First, LZD consumption in the ICU has been reduced, and it is now mainly used for targeted therapy. LZD has been replaced by vancomycin for empirical treatment of most nosocomial infections in which a Gram-positive bacterium could be involved. Moreover, as an antimicrobial stewardship intervention, since 2017, an expert assistant on infectious diseases has reviewed (on a weekly basis or on request) the use of broad-spectrum antimicrobials in the ICU. Following the implementation of these measures, both LZD consumption and the rate of LRSE decreased in 2017 to 6.34 DDDs and 13.6%, respectively.

This study highlights the importance of infection control measures and antimicrobial stewardship interventions to control the dissemination of difficult-to-treat LRSE nosocomial endemic clones, and to preserve the therapeutic efficacy of LZD.

Conflict of interest statement
None declared.

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